

**U.S.S.N. 08/786,988**

**LITTLE, et al.**

**AMENDMENT AFTER FINAL**

An executed DECLARATION pursuant to 37 C.F.R. §1.132, with an attached exhibit (Little *et al.* (1997) "MALDI on a Chip: Analysis of Arrays of Low-Femtomole to Subfemtomole Quantities of Synthetic Oligonucleotides and DNA Diagnostic Products Dispensed by a Piezoelectric Pipet", Anal chem 69:4540-4546, which is of record in this application), is submitted herewith.

Claims 1-6, 9-34, 40-51 and 54-99 are pending in this case. are pending in this application. Claims 100 and 101 are cancelled without prejudice; applicant reserves the right to file the subject matter of the cancelled claims in a divisional application.

**THE REJECTION OF CLAIMS 100 and 101 UNDER 35 U.S.C. § 112, SECOND PARAGRAPH**

Claims 100 and 101 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter that applicant regards as the invention because:

it is unclear for what they are intended for since the array of samples have been recited in the independent claims. Additionally, the product claims do not further limit the steps of the recited of the method claim.

It is respectfully submitted that this rejection has been rendered moot by cancellation of claims 100 and 101 herein.

**THE REJECTION OF CLAIMS 1-6, 9-34, 40-51, 54-69 and 70-101 UNDER 35 U.S.C. § 103(a)**

**Rejection over Tisone in view of Patterson**

Claims 1-6, 9-34, 40-51, 54-69 and 87-101 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Tisone, U.S. Patent No. 5,743,960 ("Tisone") in view of Patterson, U.S. Patent No. 5,869,240 ("Patterson") because Tisone is alleged to teach a method for dispensing a material on a substrate substantially similar to that as presently claimed. Tisone's method is alleged to comprise the steps of providing a vesicle having an interior chamber containing a fluid, disposing the vesicle adjacent to a first location on the surface of a substrate, controlling the vesicles to eject from the chamber a

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nanoliter volume of the fluid to dispense the fluid at the first location on the surface of the substrate, and moving the vesicle to a set of positions so that fluid is dispensed from the vesicle at each location of the set for forming an array of fluid material (figures 1, 6 and 7). Tisone is also alleged to teach that the method can be used to dispense sample fluids onto a diagnostic test strip for testing. It is acknowledged that Tisone does not teach or suggest the step of performing mass spectrometry analysis for the material. Nevertheless, it is alleged that such an analysis step is considered conventional in the art and is taught or suggested in Patterson. Patterson is alleged to teach a method for sequencing polymers using a mass spectrometer in order to provide a rapid, automated and cost effective sequencing of polymers with a statistical certainty.

It is concluded that it would have been obvious to one of ordinary skill in the art at the time the invention was made to have provided the method and apparatus of Tisone with a spectrometer as taught in Patterson to provide a rapid, automated and cost effective sequencing of polymers with a statistical certainty.

The rejection is respectfully traversed insofar as it applies to any the presently pending claims. It is respectfully submitted that the instant claims are not prima facie obvious over the combination of references for reasons of record. In addition, notwithstanding the failure of the Examiner to set forth a prima facie case of obviousness, a DECLARATION under 37 C.F.R. §1.132 was previously submitted.

The DECLARATION was provided to show unexpected results, which are results that are not taught or suggested by the cited art. The combination of references does not teach or suggest the unexpected results that derive from using arrays produced by dispensing nanoliter volumes of sample *for mass spectrometric analyses*. The DECLARATION shows that when small volumes as

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described in the instant application are dispensed in arrays, the resulting spots are about the size of a focussed laser spot. The resulting mass spectra (see, e.g., Figure 2 of the attached Exhibit, Little *et al.*) are uniform. They are far more uniform than spectra produced using arrays with larger spots.

As shown in the DECLARATION and the attached exhibit and in the application, when larger sample sizes (e.g., 300 nL), such as the volume used by Patterson (see, e.g., col. 11, line 56) are used, there is dramatic variability of analyte incorporation and ion yield. This results in the necessity to manually search within the resulting 1 to 2 mm diameter spots for regions from which intense signals can be obtained. This renders such arrays unamenable to automation, and also results in non-uniform spectra. The variability in resulting spectra is too great for such arrays to be used in high throughput applications, such as DNA diagnostics that require automated handling and high reproducibility.

With the smaller spots as claimed herein, searching is unnecessary, since the laser spot covers the whole spot, permitting more rapid spectrum acquisition and more quantitative and uniform results. The arrays produced by dispensing nanoliter volumes result in uniform spectra, and, hence are suitable for use in applications requiring high reproducibility.

The Examiner states that the results in the DECLARATION were not considered because it did not address the papers upon which the rejection is based. This is not appropriate. The purpose of a DECLARATION under Rule 132 is to provide factual evidence that demonstrates results that are not taught or suggested by the cited art. Discussion of the cited art is not part of a DECLARATION, but rather is the job of the attorney or agent providing the DECLARATION.

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In this instance, the primary references cited are Tisone and Ershow, each of which teaches a reagent dispensing apparatus that dispenses small volumes. Neither reference teaches or suggests using the resulting deposited material in mass spectrometric analyses. Patterson is cited for allegedly teaching mass spectrometry. Patterson teaches integrated methods and apparatus for sequencing or identifying polymers by mass spectrometry with a statistical certainty. Patterson does not teach or suggest using small spots that result from delivery of nanoliter volumes (Patterson uses spots that derived from delivery of microliter volumes; see, e.g., col. 11, line 567) nor that use of small spots is advantageous for mass spectrometric analyses. Hence the combination of cited references teaches the results achieved when mass spectrometry is performed on arrays of small spots that result from deposition of nanliter volumes.

**Use of arrays of small spots as in the instantly claimed methods results in improvements, discussed below and in the DECLARATION, in the mass spectrometric analyses that are not taught or suggested by the cited references singly or in any combination thereof.** Therefore the results achieved are by definition unexpected. It is for this purpose that the DECLARATION is provided.

**Analysis**

**The Claims**

Claims 1-6, 9-34, 40-51, 54-69 and 87-99 are directed to methods for forming an array of a sample material on surface of a substrate. The methods include the steps of: providing a vesicle that has an interior chamber containing a fluid comprising a solvent containing the sample material; disposing said vesicle adjacent to a first location of surface of a substrate without contacting the surface with the vesicle; providing mechanical pressure to the interior of the vesicle to eject from said chamber a nanoliter volume of the fluid to dispense said fluid at said first location of said surface of the substrate; moving said vesicle to each of a set of positions adjacent to the surface of the substrate,

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whereby a nanoliter volume of fluid is dispensed at each location of said set forming an array of sample material on the substrate; **and performing mass spectrometric analysis of each sample.**

Thus the method includes the step of providing mechanical pressure to the interior of the vesicle to eject from said chamber a nanoliter volume of the fluid to dispense said fluid at said first location of said surface of the substrate.

Dependent claims specify the material that is deposited, the types of vesicle and means for applying pressure, and additional method steps. For example, dependent claim 3 recites that the sample comprises a matrix material for mass spectrometry; claim 4 specifies that method further includes the step permitting the sample with matrix material is dried onto the surface; claim 5 recites that the method of claim 4 further includes adding analyte to the dried matrix to form a crystalline structure on the substrate surface. Dependent claim 6 recites that the sample comprises a matrix material for mass spectrometry and analyte.

Claims 25-30 are directed to methods for analyzing a material by mass spectrometry by dispensing nanoliter volume fluid onto surface of a substrate using the steps of: providing a vesicle comprising a fluid containing the material in a solvent; disposing the vesicle adjacent to a first location of surface of a substrate without contacting the surface with the vesicle; delivering a defined and controlled nanoliter volume of the fluid at the first location of the surface of the substrate; moving the vesicle to a second position next to the first location on the surface of the substrate to dispense a defined and controlled volume of the material along an array of locations on the substrate surface to form an array of the material; and performing mass spectrometry analysis for the material at each location of the array.

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Claims 31-34 are directed to the systems for forming an array of a sample material on surface of a substrate and for analyzing the array of sample material. The systems include a vesicle having a distal end suitable for carrying a nanoliter of fluid; a movable arm having a distal portion mounted to move the vesicle; a controller for moving the arm to dispose the vesicle adjacent to a first location on the surface of the substrate and for controlling the vesicle to provide a nanoliter volume of the fluid at the first location of the surface of the substrate; and a mass spectrometer for analyzing the material deposited on the surface of the substrate by generating a composition signal representative of the chemical composition of the material.

Claims 40-51 and 54-69 are directed to methods for dispensing nanoliter volumes of a material as an array onto the surface of a substrate, comprising the steps of: (a) providing an assembly having a plurality of vesicles arranged in the form of array for dispensing a liquid therefrom, wherein each vesicle has an interior chamber containing a fluid containing the material; (b) aligning the vesicles at a first set of locations adjacent to surface of a substrate without contacting the surface with the vesicle; (c) using mechanical pressure, controlling each of the chambers to eject a nanoliter volume of the fluid from each vesicle onto the surface of the substrate aligned with the vesicles; and (d) providing the resulting substrate with the array of material deposited thereon to mass spectrometer for determining information representative of the composition of the deposited material.

As with the other independent claims dependent claims specify the composition of the material that is deposited, the types of vesicle and means for applying pressure, and additional method steps.

Claims 91-94, specify that the mass spectrometry format is MALDI and added claims 95-99 specify that the spots are of a size that results from dispensing a volume of  $10^{-6}$  to  $10^{-10}$ .

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**The Office Action fails to set forth a case of *prima facie* obviousness**

**Relevant law**

In order to set forth a *prima facie* case of obviousness under 35 U.S.C. §103: (1) there must be some teaching, suggestion or incentive supporting the combination of cited references to produce the claimed invention (ACS Hospital Systems, Inc. v. Montefiore Hospital, 732 F.2d 1572, 1577, 221 USPQ 329, 933 (Fed. Cir. 1984)) and (2) the combination of the cited references must actually teach or suggest the claimed invention. Further, that which is within the capabilities of one skilled in the art is not synonymous with that which is obvious. Ex parte Gerlach, 212 USPQ 471 (Bd. APP. 1980). Obviousness is tested by "what the combined teachings of the references would have suggested to those of ordinary skill in the art" In re Keller, 642 F.2d 413, 425, 208 USPQ 871, 881 (CCPA 1981), but it cannot be established by combining the teachings of the prior art to produce the claimed invention, absent some teaching or suggestion supporting the combination (ACS Hosp. Systems, Inc. v Montefiore Hosp. 732 F.2d 1572, 1577, 221 USPQ 329, 933 (Fed. Cir. 1984)). "To imbue one of ordinary skill in the art with knowledge of the invention in suit, when no prior art reference or references of record convey or suggest that knowledge, is to fall victim to the insidious effect of a hindsight syndrome wherein that which only the inventor taught is used against its teacher" W.L. Gore & Associates, Inc. v. Garlock Inc., 721 F.2d 1540, 1553, 220 USPQ 303, 312-13 (Fed. Cir. 1983).

**The teachings of the cited references and differences from the claimed subject matter**

It is respectfully submitted that Tisone and Patterson, whether alone or in combination, do not render the claimed subject matter *prima facie* obvious for the reasons set forth in the previous response and incorporated herein by reference. The DECLARATION provided herewith provide results of mass spectrometric analyses performed on arrays of material deposited in accord with

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methods provided herein and compared to arrays produced with larger volumes. The comparison is provided to show the improvement.

**Tisone**

Tisone teaches a reagent dispensing apparatus including a positive displacement syringe pump in series with a solenoid valve dispenser. The pump is controlled by a stepper motor to provide an incremental quantity or continuous flow of reagent to the solenoid valve dispenser. The solenoid valve is opened and closed at a predetermined frequency and duty cycle to dispense droplets of reagent onto a target substrate at the metered flow rate.

Tisone also teaches that its apparatus can be used for aspirating ("sucking") precise quantities of reagent or other liquids from a sample or reservoir. At column 11, lines 17-21, Tisone states:

This mode may be used, for example, in a "suck and spit" operation whereby a precise quantity of fluid is aspirated from one vial containing a sample fluid and then dispensed into another vial or onto a diagnostic test strip for testing or further processing.

Tisone does not teach or even suggest, of all possible available technologies, which type of analytical technology can be used to analyze the sample transferred by the aspirating operation. Tisone certainly does not teach or even suggest that such sample can be analyzed by mass spectrometry, nor that improved performance can be achieved use of arrays produced by dispensing nanoliter volumes.

**Patterson**

Patterson does not cure this deficiency. Patterson teaches integrated methods and apparatus for sequencing or identifying polymers by mass spectrometry with a statistical certainty. The methods involve integrating data obtained by mass spectrometry analysis of a series of polymer fragments and statistically comparing the data with hypothetical data corresponding to known sequences or identities. The statistical certainty does not derive from the format subjected to mass spectrometry, but from the algorithms and

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methodology used to analyze the data. Patterson uses nanoliter volume samples for analyses (see, col. 11, line 56, - col. 12, line 8).

Patterson does not teach or suggest combining mass spectrometry analysis with arrays of samples produced by dispensing a nanovolume. Patterson does not teach or suggest that it is advantageous to perform mass spectrometry on an array of samples of a size that results from dispensing a nanoliter volume of material on a substrate. Hence, Patterson does not cure the deficiencies in the teachings of Tisone. The combination of references fails to teach or suggest the results achieved by the instantly claimed methods and systems.

**Unexpected properties**

It is impermissible to ignore the advantages, properties, utilities and unexpected results that flow from the claimed invention; they are part of the invention as a whole.

**The presently claimed methods and systems possess unexpected properties not taught or suggested by the cited references**

**THE DECLARATION OF KÖSTER**

As discussed above, the unexpected properties of the presently claimed methods are demonstrated in the DECLARATION of Köster pursuant to 37 C.F.R. §1.132. Dr. Köster and his colleagues conducted the experiments presented in the DECLARATION and in the paper Little *et al.*, which is attached to and part of the DECLARATION, as well as in examples in the application.

The results show that the sample array formed by the nanoliter dispensing methods has properties that are not taught or suggested by the cited references. Neither Tisone nor Patterson (nor Ershow), singly or in any combination thereof, teaches or suggests the results achieved when mass spectrometry is performed on an array with drops resulting from deposition of nanoliter-sized volumes.

The DECLARATION shows that spot-to-spot reproducibility from microdispensed samples is superior to that achieved using samples prepared by

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conventional pipetting, such as that used by Patterson. The DECLARATION demonstrates that the sample array formed by nanoliter volume dispensing methods having the above-described properties contributes to the shortened spectrum acquisition time (Declaration, paragraph 9), increased detection sensitivity (Declaration, paragraph 10) and makes sample handling far more routine and amenable to automation (Declaration, paragraph 11). Whe the miniaturized sample dispensing methods were used in dispensing biological samples, *e.g.*, dispensing samples generated in a temperature-cycled PROBE reaction, highly sensitive and accurate analysis could be achieved.

As a result the preparation of arrays of samples for mass spectrometric analysis, as taught in the present specification, permits highly accurate and reproducible mass spectrometric analyses to be performed. By virtue of the small spot size, there is a resulting high sample-to-sample uniformity of the sample spots, which can be entirely covered by the laser irradiation profile. This eliminates difficulties associated with nonuniform analyte incorporation and translates to a high spectrum acquisition spectrum reproducibility and high speed spectrum acquisition.

None of the cited reference singly or in combination teaches or suggests that small spot size is desirable for mass spectrometric analyses, nor that the result of such spot size leads to increased reproducibility in the results such that arrays of such spots can be used for analyses. Absent such reproducibility, arrays of samples would not be a suitable for mass spectrometric analyses, such as high throughput DNA diagnostics and sequencing.

Therefore, the presently claimed methods and systems achieve results *i.e.*, the shortened spectrum acquisition time, increased detection sensitivity, greater reproducibility, routine sample handling and amenability to automation that are not taught or suggested by the cited references.

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**The rejection of claims 70-86 and 94**

Claims 70-86 and 94 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Ershow *et al.*, which teaches a tool for dispensing small volumes, in view of Patterson, which teaches method for sequencing using a mass spectrometer. The Examiner urges that use of a mass spectrometer to analyze an array of drops, presumably prepared using the dispensing tool of Ershow *et al.* would have been conventional in the art, and hence obvious.

This rejection is respectfully traversed. As discussed in the previous response, the combination of cited references does not render the instantly claimed methods prima facie obvious. Notwithstanding this, the DECLARATION of Köster demonstrates results that are not taught or suggested the Ershow *et al.* or Patterson nor any combination thereof. Therefore, any prima facie showing of obviousness is rebutted. As above, the DECLARATION demonstrates that results are achieved when an array of spots resulting from deposition of small sized drops is used in mass spectrometric analyses.

**Claims**

Claims 70-86 and 94 are directed to methods for dispensing nanoliter volumes of a material as an array on the surface of a substrate and analyzing the material in the array by mass spectrometry, comprising the steps of: comprising the steps of: (a) providing a pin assembly having a plurality of elongated vesicles arranged as an array for dispensing a liquid therefrom, wherein each vesicle comprises a solid shaft of material having an end for retaining a nanoliter volume of fluid; (b) loading a nanoliter volume of fluid comprising a liquid material from a fluid source onto the end of the vesicles of the pin assembly; (c) disposing the pin assembly to align the vesicles at a first set of locations adjacent to surface of a substrate without contacting the surface with the vesicle; (d) dispensing the nanoliter volume of fluid without contacting the loaded fluid to the surface of the substrate aligned with the

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vesicles, whereby an array of material on the surface of the substrate is formed; and (e) analyzing the resulting arrays by mass spectrometry.

It is acknowledged that Ershow *et al.* does not teach or suggest the step of performing mass spectrometry analysis for the material. Nevertheless, it is alleged that such an analysis step is considered conventional in the art and taught in Patterson. Based on the above teachings, it is alleged that it would have been obvious to one of ordinary skill in the art at the time the invention was made to have provided the method of Ershow *et al.* with a mass spectrometer as taught by Patterson to provide a rapid, automated and cost effective sequencing of polymers with a statistical certainty.

The rejection is respectfully traversed insofar as it applies to any of claims 72-75, 77-78 and 81-83.

**Teachings of the cited references**

The teachings of Ershow *et al.* and Patterson are discussed as above. As noted above, the statistical certainty alleged to be achieved by Patterson does not derive from the format subjected to mass spectrometry, but from the algorithms and methodology used to analyze the data.

**Analysis**

It is respectfully submitted that Ershow *et al.* and Patterson, whether alone or in combination, do not render the claimed subject matter prima facie obvious. Neither reference singly or in combination teaches the unexpected results achieved when arrays produced by dispensing nanoliter volumes are used in mass spectrometric analysis.

As discussed above and shown in the application, and the attached DECLARATION, the use of such arrays in mass spectrometric analyses results in decreased spectrum acquisition times, permits automation of the processes, and results in highly uniform and reproducible spectra. Such results are not taught or suggested in the cited references. The high reproducibility achieved using the instantly claimed methods does not derive from the paradigm used to analyze

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the data as in Patterson, but from the format in which the mass spectrometry analyses are performed. Therefore, the claimed subject matter is not prima facie obvious over Ershow *et al.* and Patterson, singly or in any combination thereof.

**Rebuttal to comments made by the Examiner:**

The Examiner urges that the claims are unobvious over the combination of teachings of Tisone *et al.* or Ershow *et al.* and Patterson, because each of Tisone *et al.* and Ershow *et al.* teaches methods for dispensing small volumes and Patterson teaches mass spectrometry analyses. The Examiner states without any evidentiary support that:

. . . one of ordinary skill in the art would recognize that such a low volume sample dispensing offers several advantages such as dispensed volume accuracy and uniformity, as well as elimination of wasting expensive samples or reagents, etc.

First, it is noted that no references supporting this statement are provided. The Examiner is reminded that MPEP 2144.03 states:

The Examiner may take official notice of facts outside of the record which are capable of instant and unquestionable demonstration as being "well-known" in the art. In re Ahlert, 424 F.2d 1088, 1091, 165 USPQ 418, 420 (CCPA 1970). . . .

The above-noted properties stated by the Examiner as being known, are not "capable of instant and unquestionable demonstration as being "well-known" in the art.

MPEP 2144.03 continues:

If justified, the examiner should not be obliged to spend time to produce documentary proof. If the knowledge is of such notorious character that official notice can be taken, it is sufficient so to state. In re Malcolm, 129 F.2d 529, 54 USPQ 235 (CCPA 1942). If the applicant traverses such an assertion the examiner should cite a reference in support of his or her position.

If this position is maintained the Examiner must provide a reference supporting this position.

Second, and more significantly, the DECLARATION is not being provided to show "low volume sample dispensing offers several advantages such as

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dispensed volume accuracy and uniformity, as well as elimination of wasting expensive samples or reagents". Rather the DECLARATION is provided to show that mass spectrometric analyses that are performed on arrays that result from deposition of small volumes are more uniform and spot-to-spot reproducibility from microdispensed samples is superior to that achieved using samples prepared by conventional pipetting, such as that used by Patterson. The DECLARATION demonstrates that the sample array formed by nanoliter volume dispensing methods having the above-described properties contributes to the shortened spectrum acquisition time (Declaration, paragraph 9), increased detection sensitivity (Declaration, paragraph 10) and makes sample handling far more routine and amenable to automation (Declaration, paragraph 11). Whe the miniaturized sample dispensing methods were used in dispensing biological samples, e.g., dispensing samples generated in a temperature-cycled PROBE reaction, highly sensitive and accurate analysis could be achieved.

As a result the preparation of arrays of samples for mass spectrometric analysis, as taught in the present specification, permits highly accurate and reproducible mass spectrometric analyses to be performed. By virtue of the small spot size, there is a resulting high sample-to-sample uniformity of the sample spots, which can be entirely covered by the laser irradiation profile. This eliminates difficulties associated with nonuniform analyte incorporation and translates to a high spectrum acquisition spectrum reproducibility and high speed spectrum acquisition.

These results are not taught or suggested by any of Ershow *et al.*, Patterson *et al.* or Tisone singly or in any combination thereof. Therefore, the Examiner has failed to set forth a prima facie case of obviousness. In view of the unequivocal showing of unexpected results shown in the specification, a Notice of Appeal is filed herewith.

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In view of the above remarks and the amendments and remarks of record, consideration and allowance of the application are respectfully requested.

Respectfully submitted,  
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